INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

IDENTIFICATION OF PHYTO CONSTITUENTS AND DEVELOPMENT OF TLC AND HPTLC FINGER PRINT PROFILES FOR *EPALTES PYGMAEA* DC.

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Article Received on: 27/09/19 Approved for publication: 12/10/19

DOI: 10.7897/2230-8407.1010308

ABSTRACT

Objective of the present work was to establish of the finger print profile of five isolated compounds namely *β*-amyrin acetate, *β*-amyrin, lupeol, stigmasterol, β-sitosterols in *Epaltes pygmaea* DC using thin layer chromatography and high performance thin layer chromatography (TLC/HPTLC) techniques. β-amyrin acetate, β-amyrin, lupeol, stigmasterol, β-sitosterols, *n*-hexane, chloroform, ethyl acetate and ethanol extract of the *Epaltes pygmaea* whole plant were applied on a TLC plate, developed using Toluene: Ethyl acetate as solvent system and visualized under UV 254 nm and *366*nm for photo documentation. The HPTLC finger printing of the n-hexane, chloroform, ethyl acetate, ethanol extracts were developed in the visible light at 520 nm. Presence of β-amyrin acetate, β-amyrin, lupeol, stigmasterol, β-sitosterols were confirmed by UV spectra comparison of compound and respective peaks of similar R_f in extract. TLC and HPTLC finger print profile one of the useful tool in compound identification and authentication in medicinal plants.

Keywords: *Epaltes pygmaea*, TLC/HPTLC, amyrin, lupeol, stigmasterol, β-sitosterols.

INTRODUCTION

Epaltes is used in traditional Ayurvedic medicine in Sri Lanka to alleviate jaundice. Literature survey reveals that the plant of the genus has the therapeutic action of diaphoretics, diuretics; stimulant and expectorant are used in urethral discharges and acute dyspepsia[1]. The genus *Epaltes* of the family Asteraceae is found in the tropics. About nine species are recorded in the world wide; only about two species are reported in India[2-4]. Alcohol and aqueous extract of *E. pygmaea* possesses hepatoprotective activity against paracetamol-induced liver damage in rats and also have potent diuretic activity[5]. Recent studies show that the extract of *E. pygmaea* has good inhibitory activity against the organism *Bacillus cereus, Klebsiella pneumonia* and *Staphylococcus aureus* at microgram level[6]. Microscopic, thin layer chromatographic studies[7] and quality control parameters[8] of *E. pygmaea* have been reported. The chemical constituents of this plant were lupeol acetate, stigmasterol, stigmasterol acetate, apigenin, luteolin, apigenin-7-O-glucoside and luteolin-7-O-glucoside[9]. A complete review of literature from various sources revealed that detailed work has not been carried out in *E. pygmaea* particularly chemical studies. The present study includes isolation of phytoconstituents, characterization and TLC photo documentation and HPTLC finger print profiles with successive *n*-hexane, chloroform, ethyl acetate and ethanol extract along with isolated phytoconstituents for the identification of this plant.

MATERIAL AND METHODS

Plant material

Fresh whole plant of *Epaltes pygmaea* DC. collected from Tirunelveli District was identified and authenticated by Prof. P. Jeyaraman, Director, Institute of Herb Botany, Plant Anatomy Research Centre, Tambaram, Chennai, India. A voucher specimen no. PARC/2014/2071 was obtained for the submitted sample. Plant was shade dried and powdered in a pulverizer.

Instrumentation

CAMAG HPTLC system equipped with a sample applicator TLC Auto sampler 4, twin trough plate development chamber, Scanner IV, Visualizer, win CATS software version 1.4.4.

Material and Reagents

AR grade n-hexane, chloroform, ethyl acetate, ethanol and toluene were obtained from E. Merck, India. LR grade vanillin and sulphuric acid were obtained from SRL chemicals.

TLC/ HPTLC Profiles

TLC/HPTLC studies were carried out following the method of Sethi, Stahl and Wagner[10-12].

Sample preparation

The plant material was successively extracted with *n*-hexane, chloroform, ethyl acetate and ethanol solvents by cold percolation method of extraction. All the obtained extracts were filtered using Whatman filter paper no.2, dried using Rotavapor R-300 and stored separately for further use.

Developing solvent system

The mobile phase used for developing the successive extracts of the plant and isolated compounds β-amyrin acetate, β-amyrin, lupeol, stigmasterol, β-sitosterols was Toluene: Ethylacetate (10:1).

Sample application

The samples were spotted in the form of bands of width 7 mm on an aluminium TLC plate precoated with Silica gel $60F_{254}$ (E. Merck) of 0.2 mm thickness with the help of TLC Auto sampler 4, which is programmed through win CATS software version 1.4.4. Applied volume of four extracts of *E. Pygmaea* was 15 µl and each isolated phytoconstituents was 3 µl.

Development of chromatogram and photo documentation

Development of the plate up to a migration distance 80 mm was performed at 29 ± 2°C with the mobile phase for each extracts in a twin trough chamber previously saturated for 30 min. Extract 15 µl and 3 µl the sample were applied. After development, the plate was air dried, viewed under UV 254 nm and 366 nm and the image were documented.

Detection of spot scanning

The developed plate was then derivatized in vanillin sulphuric acid reagent and dried at 105°C in hot plate till the appearance of colour of the band. The plate was kept in visualizer photo documentation in white light. Then the plate was scanned at 520 nm using the scanner 4 equipped with win CATS software version 1.4.4 using tungsten lamp as light source with the slit dimension of 6 x 0.45 mm. The R_f values, 3D chromatogram and spectral comparison were recorded.

RESULT AND DISCUSSION

The TLC profile of successive *n*-hexane, chloroform, ethyl acetate, ethanol of extracts of *E. pygmaea* and isolated marker compounds of *β*-amyrin acetate, *β*-amyrin, lupeol, stigmasterol, *β*-sitosterols under UV 254 nm, 366 nm and visible light after derivatization with vanillin sulphuric acid regent are showed in Figure 1.

The TLC photo documentation of 15 µl of *n*-hexane extract of *E. pygmaea*, 9 spot appeared under UV 254 nm at R_f 0.04, 0.10, 0.15, 0.20, 0.25, 0.42, 0.46, 0.56, 0.74 (all green colour). Under 366 nm, the TLC plate showed 11 different components at R_f 0.13 (red), 0.21 (red), 0.35 (blue), 0.42 (red), 0.52 (green), 0.57 (red), 0.61 (blue), 0.63 (red), 0.71 (blue) 0.81 (blue), 0.89 (red). The TLC plate in visible light after derivatization with vanillin-sulphuric acid reagent showed 12 different spot sat R_f 0.14, 0.20, 0.24, 0.31, 0.39, 0.46, 0.54, 0.65, 0.72, 0.91, 0.95, 0.97 (all spots in violet colour) (Figure 1).

The TLC profile of chloroform extract of *E. pygmaea* under UV 254 nm, UV 366 nm and visible light after derivatization with vanillin-sulphuric acid reagent showed 6, 8 and 9 spot different components respectively. The R_f and are 0.05,0.24, 0.42, 0.57, 0.65,0.75 (all spot green) at 254 nm; 0.11 (reddish violet), 0.20 (red), 0.40 (red), 0.44 (red), 0.51 (light green), 0.57 (reddish violet), 0.71 (blue),0.82 (blue) at 366 nm; 0.14, 0.23, 0.29, 0.40, 0.42, 0.55, 0.65, 0.92, 0.94 (all spots in violet colour) at 520 nm (Figure 1).

The TLC profile of ethyl acetate extracts of *E. pygmaea* under UV 254 nm, UV 366 nm and visible light after derivatization with vanillin-sulphuric acid reagent showed in 5, 7 and 7 spots. The R_f values are 0.11, 0.26, 0.43, 0.57, 0.74 (all spots in green colour) under 254 nm; 0.06 (red), 0.16 (reddish blue), 0.29 (red), 0.42 (red), 0.51 (light green), 0.58 (reddish violet), 0.69 (red) under 366 nm; 0.14, 0.24, 0.29, 0.40, 0.55, 0.65, 0.94 (all spots in violet colour) at 520 nm.

The TLC profile of ethanol extracts of *E. pygmaea* under UV 254 nm, UV 366 nm and visible light after derivatization with vanillin-sulphuric acid reagent showed in 6, 6 and 7 spots. The R_f values are 0.10, 0.16, 0.26, 0.43, 0.57, 0.74 (all spots in green colour) under 254 nm; under 366 nm 0.12 (reddish violet), 0.22 (red), 0.29 (red), 0.41 (red), 0.51 (light green), 0.57 (reddish violet); 0.12, 0.24, 0.29, 0.40, 0.55, 0.65 and 0.94 (all spots in violet colour) at 520 nm.

In the TLC profile, *β*-amyrin acetate appeared at R_f 0.92, lupeol at 0.53, *β*-amyrin at 0.52, stigmasterol 0.39 and *β*-sitosterols at 0.38 under visible light after derivatization with vanillin sulphuric acid reagent. All these spots are found to be prominent in appearance in low polar solvents when compared with high polar solvent and the intensity of the spots decrease from *n*-hexane to ethanol. In the HPTLC finger print profile of *n*-hexane, chloroform, ethyl acetate and ethanol extract of *E. pygmaea*, out of 19, 18, 16 and 15 components, the peaks of β-amyrin acetate varied with R_f values from 0.94 to 0.92 (9.09%, 11.63%, 12.781% and 22.45); the peaks of lupeol/β-amyrin 0.53/0.52 got merged together either at 0.53 or at 0.52 (13.15%, 11.64%, 9% and 13.1%); similarly the peak of stigmasterol/*β*-sitosterols at 0.39/0.40 got merged together either at 0.39 or at 0.40 (area 9.75%, 6.16%, 14.05% and 10.56%) were found to be prominent respectively in the successive extracts. Since the peaks of lupeol/β-amyrin and stigmasterol/*β*-sitosterols merged together, the quantification of these compounds is not possible with HPTLC technique. However the presence is confirmed with their UV spectra comparison of respective compound with the compounds of similar R_f from the extracts. Other components were found to be very less in area percentage and thereby their individual content also (Figure 2 and 3).

The HPTLC chromatogram, UV spectral comparison and fingerprint profile of all compounds and extracts (*β*-amyrin acetate, *β*-amyrin, lupeol, stigmasterol, *β*-sitosterols) were shown in Figures 4-8.

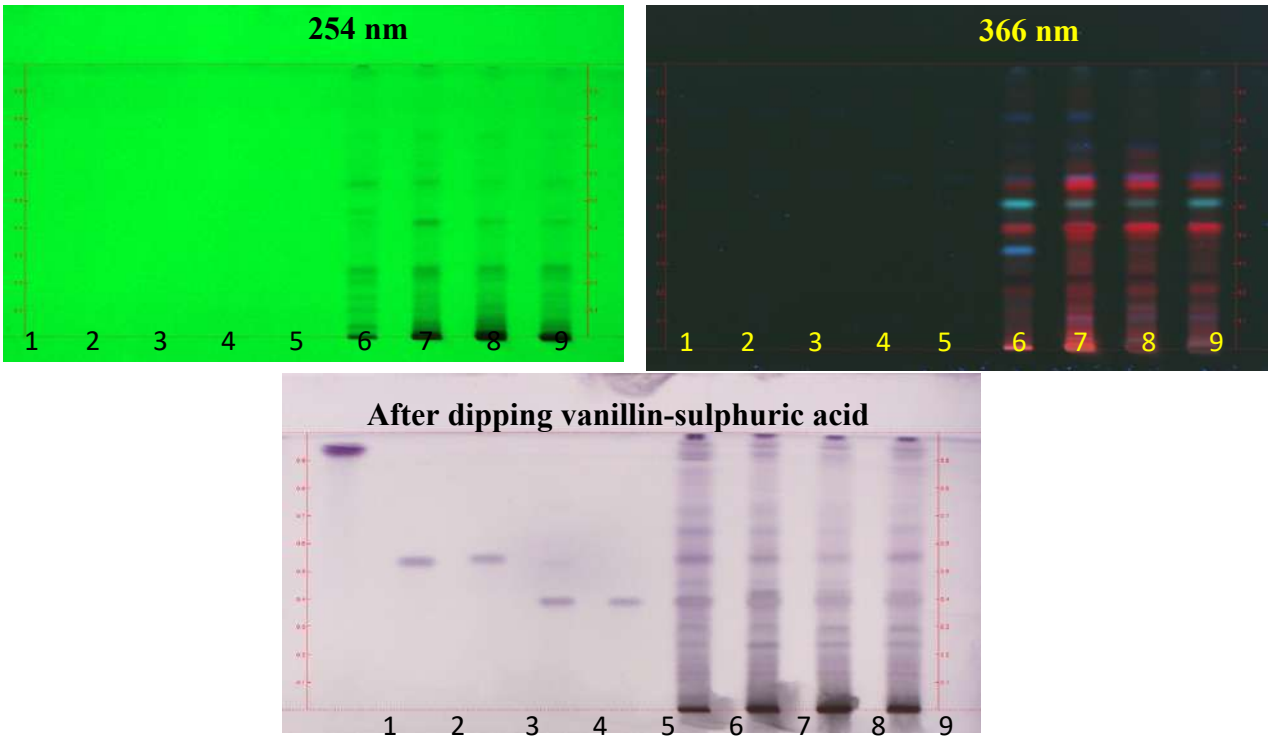
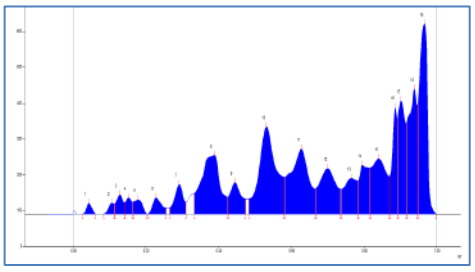


Figure 1: TLC Photodcocumentation of different extracts of *E. pygmaea*

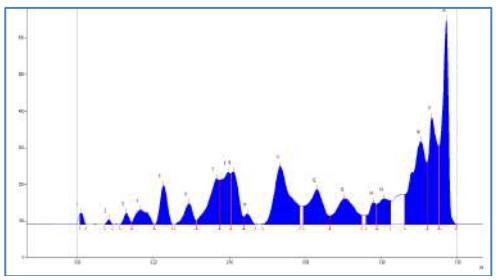
Track 1: *β*-Amyrin acetate, **Track 2**: *β*-Amyrin, **Track 3**: Lupeol, **Track 4**: Stigmasterol, **Track 5**: β-Sitosterol, **Track 6**: Hexane extract, **Track 7**: Chloroform extract, **Track 8**: Ethyl acetate extract, **Track 9**: Ethanol extract.

Thus the developed chromatogram for *E. pygmaea* is specific with the selected solvent system Toluene: Ethyl acetate (10:1, v/v). The TLC R_f values and characteristic HPTLC finger print profiling along with marker chemical compounds of any plant is helpful in the identification of any species. Thus the present study provides sufficient information about identification of phyto constituents of this plant which in turn serves as a reference data for the standardization and quality control of this medicinal plant.



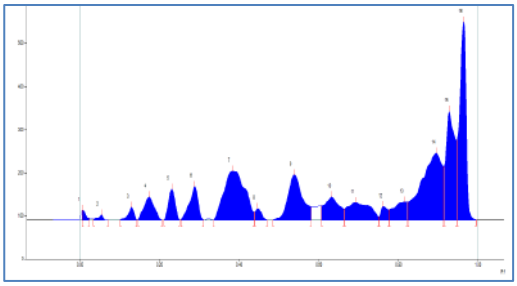
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	9.6 AU	0.04 Rf	29.9 AU	0.96 %	0.06 Rf	0.1 AU	376.1 AU	0.49 %
2	0.08 Rf	0.1 AU	0.11 Rf	38.6 AU	1.02 %	0.11 Rf	28.1 AU	395.7 AU	0.52 %
3	0.11 Rf	28.4 AU	0.13 Rf	53.3 AU	1.77 %	0.14 Rf	30.7 AU	887.1 AU	1.14 %
4	0.14 Rf	31.3 AU	0.15 Rf	45.2 AU	1.50 %	0.16 Rf	33.9 AU	658.4 AU	0.86 %
5	0.17 Rf	33.4 AU	0.18 Rf	39.5 AU	1.31 %	0.20 Rf	0.5 AU	799.3 AU	1.05 %
6	0.21 Rf	0.2 AU	0.23 Rf	44.8 AU	1.49 %	0.26 Rf	18.1 AU	983.5 AU	1.29 %
7	0.27 Rf	17.8 AU	0.29 Rf	82.3 AU	2.73 %	0.31 Rf	32.2 AU	1747.0 AU	2.29 %
8	0.33 Rf	57.9 AU	0.36 Rf	163.8 AU	5.44 %	0.45 Rf	47.1 AU	7426.5 AU	9.75 %
9	0.43 Rf	47.7 AU	0.45 Rf	87.8 AU	2.92 %	0.47 Rf	42.5 AU	2244.0 AU	3.05 %
10	0.48 Rf	42.6 AU	0.53 Rf	243.1 AU	8.07 %	0.58 Rf	82.4 AU	10018.4 AU	13.15 %
11	0.58 Rf	102.5 AU	0.63 Rf	181.6 AU	6.03 %	0.67 Rf	76.2 AU	7979.6 AU	10.47 %
12	0.67 Rf	76.7 AU	0.70 Rf	127.2 AU	4.22 %	0.74 Rf	69.9 AU	5105.9 AU	6.70 %
13	0.74 Rf	69.9 AU	0.77 Rf	99.2 AU	3.29 %	0.78 Rf	91.8 AU	3111.8 AU	4.08 %
14	0.79 Rf	92.8 AU	0.80 Rf	136.7 AU	4.54 %	0.82 Rf	28.7 AU	3128.1 AU	4.10 %
15	0.82 Rf	128.7 AU	0.84 Rf	154.1 AU	5.12 %	0.87 Rf	96.2 AU	5386.4 AU	7.07 %
16	0.87 Rf	106.1 AU	0.89 Rf	297.2 AU	9.87 %	0.89 Rf	66.8 AU	3066.5 AU	4.81 %
17	0.89 Rf	272.6 AU	0.90 Rf	315.7 AU	10.49 %	0.92 Rf	52.0 AU	5175.6 AU	6.79 %
18	0.92 Rf	255.2 AU	0.94 Rf	348.9 AU	11.59 %	0.95 Rf	93.2 AU	6818.1 AU	9.06 %
19	0.95 Rf	319.1 AU	0.97 Rf	536.1 AU	17.81 %	1.00 Rf	0.0 AU	10114.0 AU	13.27 %

A



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	28.0 AU	0.01 Rf	30.5 AU	1.38 %	0.02 Rf	0.4 AU	254.4 AU	0.52 %
2	0.07 Rf	0.4 AU	0.08 Rf	12.2 AU	0.54 %	0.09 Rf	0.3 AU	109.0 AU	0.22 %
3	0.11 Rf	0.2 AU	0.13 Rf	30.8 AU	1.36 %	0.14 Rf	5.3 AU	373.9 AU	0.76 %
4	0.14 Rf	5.6 AU	0.17 Rf	39.7 AU	1.77 %	0.20 Rf	0.6 AU	1146.4 AU	2.34 %
5	0.20 Rf	0.6 AU	0.23 Rf	106.6 AU	4.74 %	0.25 Rf	0.7 AU	1735.3 AU	3.54 %
6	0.26 Rf	0.2 AU	0.29 Rf	56.7 AU	2.52 %	0.31 Rf	10.7 AU	1138.7 AU	2.32 %
7	0.31 Rf	11.0 AU	0.37 Rf	124.4 AU	5.53 %	0.37 Rf	21.0 AU	2945.3 AU	6.01 %
8	0.37 Rf	121.1 AU	0.40 Rf	142.4 AU	6.33 %	0.40 Rf	38.7 AU	3029.4 AU	6.18 %
9	0.41 Rf	139.1 AU	0.41 Rf	143.4 AU	6.35 %	0.44 Rf	29.7 AU	2358.9 AU	4.81 %
10	0.44 Rf	29.8 AU	0.45 Rf	28.6 AU	1.25 %	0.47 Rf	0.6 AU	416.0 AU	0.85 %
11	0.48 Rf	1.2 AU	0.53 Rf	158.6 AU	7.05 %	0.59 Rf	51.4 AU	5704.4 AU	11.64 %
12	0.59 Rf	50.5 AU	0.63 Rf	95.4 AU	4.24 %	0.66 Rf	23.6 AU	3278.6 AU	6.69 %
13	0.67 Rf	23.7 AU	0.70 Rf	70.1 AU	3.12 %	0.75 Rf	25.7 AU	2998.0 AU	6.12 %
14	0.76 Rf	25.4 AU	0.78 Rf	80.2 AU	2.88 %	0.79 Rf	55.1 AU	991.2 AU	2.02 %
15	0.79 Rf	55.4 AU	0.81 Rf	89.5 AU	3.09 %	0.83 Rf	84.8 AU	1822.7 AU	3.72 %
16	0.86 Rf	82.0 AU	0.90 Rf	226.3 AU	10.05 %	0.92 Rf	57.6 AU	7153.0 AU	14.65 %
17	0.92 Rf	165.4 AU	0.93 Rf	282.5 AU	13.01 %	0.95 Rf	13.7 AU	5700.9 AU	11.63 %
18	0.95 Rf	215.4 AU	0.97 Rf	560.3 AU	24.92 %	1.00 Rf	0.7 AU	7839.8 AU	15.99 %

B



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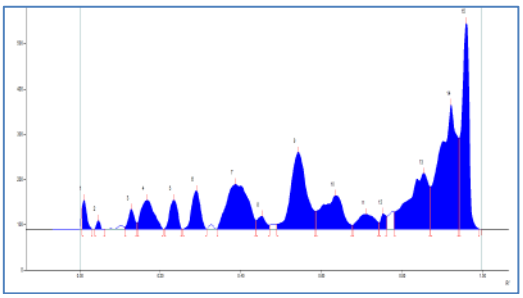
2	0.03 Rf	2.2 AU	0.05 Rf	12.8 AU	0.82 %	0.07 Rf	0.3 AU	142.0 AU	0.38 %
3	0.10 Rf	0.1 AU	0.13 Rf	29.4 AU	1.89 %	0.14 Rf	0.2 AU	384.6 AU	1.02 %
4	0.15 Rf	0.9 AU	0.17 Rf	54.0 AU	3.48 %	0.21 Rf	0.1 AU	1243.7 AU	3.30 %
5	0.21 Rf	0.5 AU	0.23 Rf	72.7 AU	4.68 %	0.25 Rf	0.0 AU	1061.4 AU	2.82 %
6	0.25 Rf	0.7 AU	0.29 Rf	78.5 AU	5.05 %	0.31 Rf	0.2 AU	1492.7 AU	3.96 %
7	0.34 Rf	0.1 AU	0.39 Rf	113.0 AU	7.28 %	0.44 Rf	18.7 AU	5298.6 AU	14.05 %
8	0.44 Rf	19.0 AU	0.45 Rf	26.8 AU	1.72 %	0.47 Rf	0.4 AU	378.5 AU	1.00 %
9	0.49 Rf	0.1 AU	0.54 Rf	105.4 AU	6.78 %	0.58 Rf	30.3 AU	3392.2 AU	9.00 %
10	0.61 Rf	34.1 AU	0.63 Rf	54.6 AU	3.52 %	0.67 Rf	26.0 AU	1753.3 AU	4.65 %
11	0.67 Rf	26.4 AU	0.69 Rf	41.1 AU	2.65 %	0.75 Rf	7.8 AU	2053.3 AU	5.45 %
12	0.75 Rf	8.3 AU	0.76 Rf	31.6 AU	2.03 %	0.78 Rf	24.2 AU	465.3 AU	1.23 %
13	0.78 Rf	24.5 AU	0.82 Rf	42.1 AU	2.71 %	0.82 Rf	41.7 AU	1228.1 AU	3.26 %
14	0.83 Rf	41.8 AU	0.90 Rf	155.1 AU	9.99 %	0.92 Rf	23.9 AU	7094.4 AU	18.82 %
15	0.92 Rf	124.8 AU	0.93 Rf	252.4 AU	16.25 %	0.95 Rf	84.8 AU	4830.9 AU	12.81 %
16	0.95 Rf	188.8 AU	0.97 Rf	459.5 AU	29.59 %	1.00 Rf	0.3 AU	6687.3 AU	17.74 %

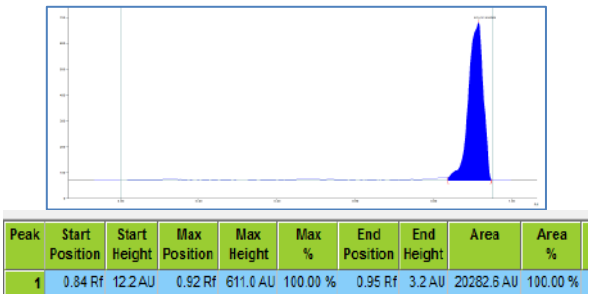
C



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	47.2 AU	0.01 Rf	84.9 AU	3.97 %	0.03 Rf	0.2 AU	590.3 AU	1.34 %
2	0.04 Rf	0.2 AU	0.05 Rf	19.7 AU	1.21 %	0.06 Rf	0.3 AU	148.6 AU	0.34 %
3	0.11 Rf	3.7 AU	0.13 Rf	43.5 AU	2.67 %	0.14 Rf	13.2 AU	587.2 AU	1.34 %
4	0.14 Rf	14.3 AU	0.17 Rf	84.0 AU	3.92 %	0.21 Rf	0.0 AU	1796.8 AU	4.09 %
5	0.21 Rf	0.2 AU	0.23 Rf	84.8 AU	3.96 %	0.25 Rf	0.4 AU	1093.9 AU	2.49 %
6	0.26 Rf	0.1 AU	0.29 Rf	84.5 AU	5.17 %	0.32 Rf	0.1 AU	1565.5 AU	3.56 %
7	0.34 Rf	0.4 AU	0.39 Rf	99.5 AU	6.09 %	0.44 Rf	19.3 AU	4642.0 AU	10.56 %
8	0.44 Rf	19.4 AU	0.45 Rf	28.8 AU	1.76 %	0.47 Rf	8.5 AU	502.5 AU	1.14 %
9	0.49 Rf	10.5 AU	0.54 Rf	169.9 AU	10.40 %	0.59 Rf	39.5 AU	5755.2 AU	13.10 %
10	0.59 Rf	39.7 AU	0.64 Rf	73.7 AU	4.51 %	0.68 Rf	8.1 AU	3227.1 AU	7.34 %
11	0.68 Rf	8.1 AU	0.71 Rf	33.2 AU	2.03 %	0.74 Rf	13.7 AU	1212.6 AU	2.76 %
12	0.75 Rf	14.3 AU	0.75 Rf	34.3 AU	2.10 %	0.76 Rf	29.0 AU	420.2 AU	0.96 %
13	0.76 Rf	36.0 AU	0.86 Rf	123.3 AU	7.55 %	0.87 Rf	92.5 AU	5445.3 AU	12.39 %
14	0.87 Rf	92.6 AU	0.92 Rf	274.9 AU	16.83 %	0.94 Rf	60.2 AU	9864.7 AU	22.45 %
15	0.94 Rf	200.4 AU	0.96 Rf	454.8 AU	27.85 %	0.99 Rf	0.5 AU	7087.2 AU	16.13 %

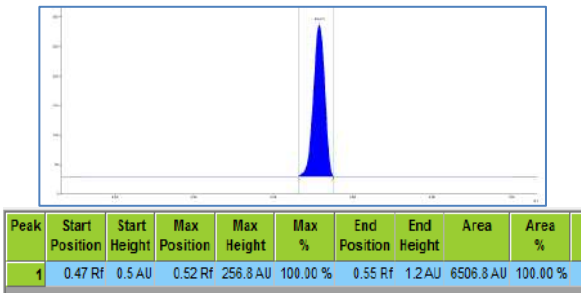
D

Figure 2: **HPTLC finger print profiles and peak tables of *E. pygmaea* at 520 nm**
A. Hexane extract; **B**. Chloroform extract; **C**.Ethyl acetate extract; **D**. Ethanol extract



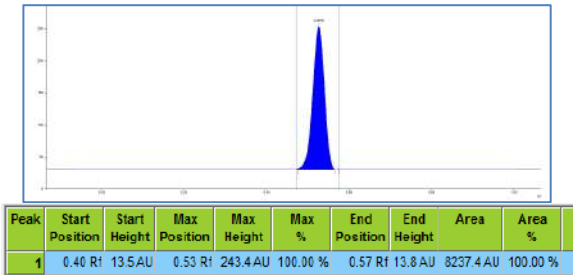
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.84 Rf	12.2 AU	0.92 Rf	611.0 AU	100.00 %	0.95 Rf	3.2 AU	20282.6 AU	100.00 %

A



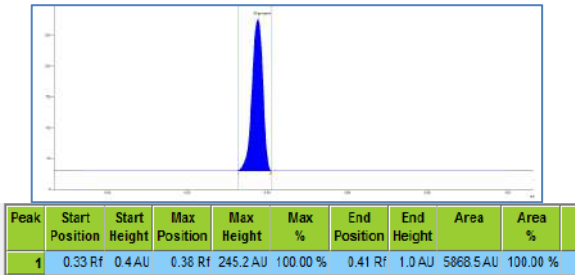
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.47 Rf	0.5 AU	0.52 Rf	256.8 AU	100.00 %	0.55 Rf	1.2 AU	6506.8 AU	100.00 %

B



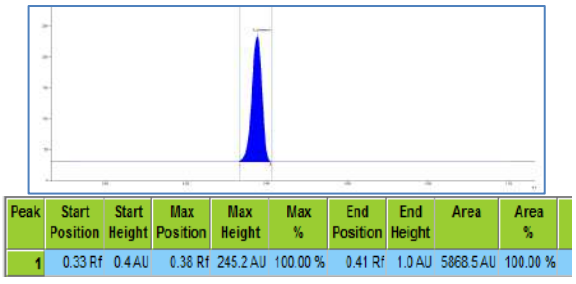
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.40 Rf	13.5 AU	0.53 Rf	243.4 AU	100.00 %	0.57 Rf	13.8 AU	8237.4 AU	100.00 %

C



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.33 Rf	0.4 AU	0.38 Rf	245.2 AU	100.00 %	0.41 Rf	1.0 AU	5868.5 AU	100.00 %

D



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.33 Rf	0.4 AU	0.38 Rf	245.2 AU	100.00 %	0.41 Rf	1.0 AU	5868.5 AU	100.00 %

E

Figure 3: HPTLC finger print profiles and peak tables of phyto constituents
A. *B*-amyrin acetate; **B**. *β*-amyrin; **C**. Lupeol; **D**. Stigmasterol; **E**. *β*-sitosterol

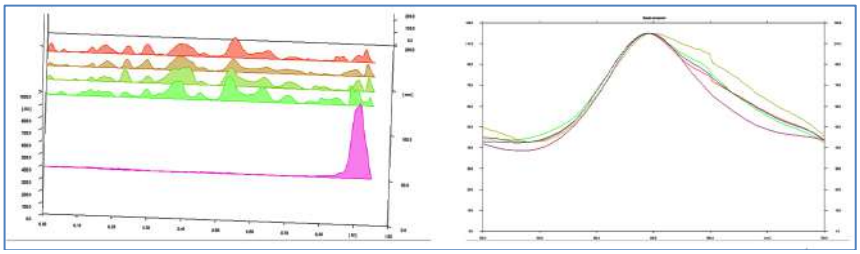


Figure 4: 3D Chromatogram and UV Spectral comparison of *β*-amyrin acetate with corresponding spot of different extracts of *E. pygmaea*

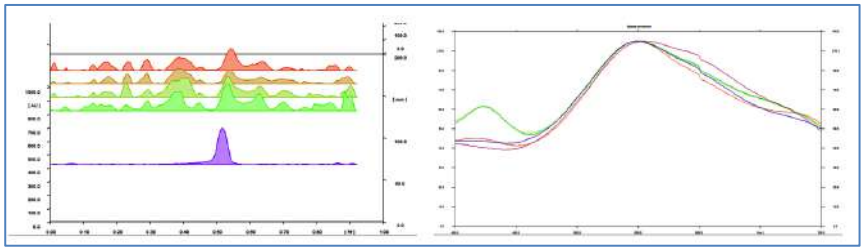


Figure 5: 3D Chromatogram and UV Spectral comparison of *β*-amyrin with corresponding spot of different extracts of *E. pygmaea*

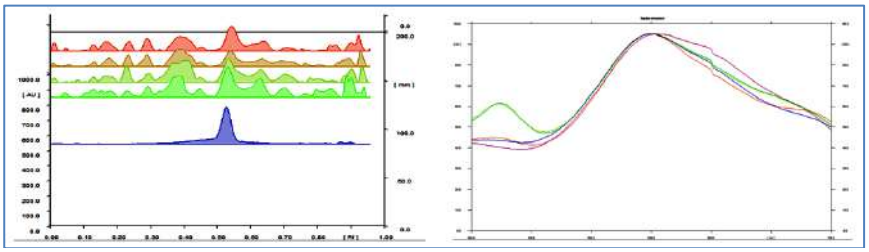


Figure 6: 3D Chromatogram and UV Spectral comparison of lupeol with corresponding spot of different extracts of *E. Pygmaea*

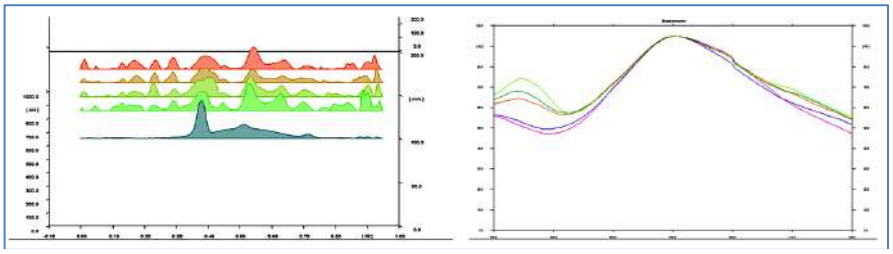


Figure 7: 3D Chromatogram and UV Spectral comparison of stigmasterol with corresponding spot of different extracts of *E. pygmaea*

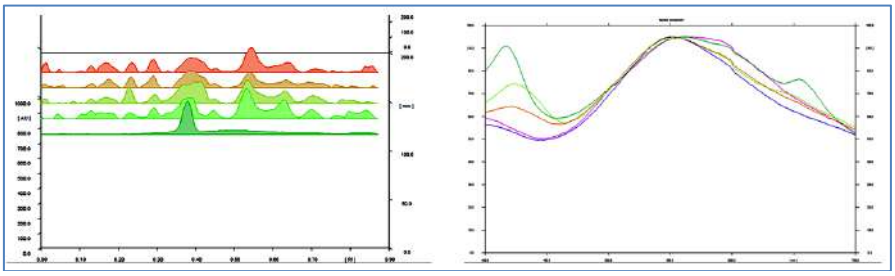


Figure 8: 3D Chromatogram and UV Spectral comparison of *β*-sitosterol with corresponding spot of different extracts of *E. pygmaea*

CONCLUSION

In conclusion, the results obtained from TLC photo documentation along with phyto chemical constituents and evaluation of HPTLC fingerprint profiling is helpful in the identification of *Epaltes pygmaea* DC. whole plantand quality control of the herbal drug formulation in which this plant is an ingredient; for the estimation of these compounds in this plant and other herbal preparations and may be attempted with other chromatographic technique.

ACKNOWLEDGEMENT

The authors are thankful to Director General, CCRAS, Director General, CCRS and Assistant Director I/c, SCRI, Chennai for providing necessary facilities and support.

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Cite this article as:

S. Murugammal *et al*. Identification of Phyto constituents and Development of TLC and HPTLC finger print profiles for *Epaltes pygmaea* DC. Int. Res. J. Pharm. 2019; 10(10):109-114 http://dx.doi.org/10.7897/2230-8407.1010308

Source of support: Nil, Conflict of interest: None Declared

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